International Journal of Agricultural
Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 4, Issue 5, Oct 2014, 55-66


# BIOLOGICAL CONTROL OF DAMPING OFF AND STEM ROT OF TOMATO *(Lycopersicon esculentum Mill.)* USING AN ANTAGONISTIC ACTINOMYCETE, *SACCHAROPOLYSPORA SP.*

**PRABHA S. B, VIGNESH A & K. MURUGESAN**

Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, Tamil Nadu, India

## ABSTRACT

Actinomycetes have been found to protect plants against their diseases. Stem rot and damping off of tomato caused by *Sclerotinia sclerotiorum* and *Rhizoctonia solani*, respectivelyare one of the major yield-limiting factor for tomato production. The present study aimed at the isolation of actinomycetes from different rhizosphere soil of tomato plants and to check their antagonistic effect against two dreadful phytopathogenic fungi such as *Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

The selected isolate was further identified and characterized using physiochemical and biochemical methods. Fifty strains were isolated and the strain AMP14 showed significant activity against the two fungi, *R.solani* and *S.sclerotiorum* on preliminary screening by dual culture plate method as well as crude was also checked for its antagonistic effect. Further for the species level identification of the actinomycete, AMP14, was identified using 16srDNA sequencing was used and found to be *Saccharopolyspora erythraea*.

**KEYWORDS:** Biological Control, *Saccharopolyspora erythraea*, Phytopathogens

## INTRODUCTION

Actinomycetes are well known Gram positive organisms mainly because of their chemical and morphological diversity (Goodfellow and Donnewell, 1989)

Actinomycetes, produce several secondary metabolites that have high DNA, G+C content. Besides, they also produce antibiotics which affects fungal growth (Goodfellow and Williams, 1983). Their metabolite formed potential applications in pharmacy, industry, agriculture and environment. The ability of chitinase production enhances their synergistic effect by having chitin as main component of cell wall (Goodfellow *et al*., 1988).

Actinomycetes prefer to grow in soil constituents such as humus, litter, dung and even on rock surfaces and are ubiquitous in nature (Lechevalier, 1981). Over 20 genera have been isolated from soil and viable counts of several millions per gram soil are common (Williams and Wellington, 1982).

Tomato is one of the important and popular vegetable crops in India. Many diseases and disorders can affect tomatoes during the growing season. In India it is grown in 4.58 million hectares with a production of 74.62 million tones per season.

Biological control of plant diseases, especially due to fungal infection have been achieved using antagonistic micro organisms such as *Trichoderma sp, Pseudomonas sp, Bacillus sp and Streptomyces sp*. (Elad *et al*., 1980).

*R.solani* is one of the dreadful phytopathogens that attack tomatoes cultivated Woldwide causing root and crown rot (Latorre, 2004).

Soil borne pathogens are complex not only in their biochemical constituents but also in their behavioural pattern. *R. solani* is a plant pathogenic fungus with a wide host range and worldwide distribution. It is the major fungus responsible for damping-off, black spot and root rot diseases (Neha and Dawande, 2010) of several economically important crops throughout the world, causing severe yield loss.

The fungus *S. sclerotiorum* causes a stem rot disease condition in tomato plants called as timber rot. This fungus attacks a wide host range of plants such as beans, cabbage, carrot, celery, cucumbers, lettuce, onions, peas, pumpkins and squash.

*S. sclerotiorum* is a non-host specific necrotrophic fungal pathogen, which is the casual agent of stem rot and could attack 400 species worldwide and is considered as one of the serious threat to many economically important crops. (Boland and Hall, 1994; Hegedus and Rimmer., 2005).

## MATERIALS AND METHODS

### Sample Collection

Soil samples were collected from 5-15cm depth into sterile plastic bags from different rhizosphere soil of tomato fields such as Kancheepuram, Villupuram and Dharmapuri districts and brought to the laboratory in aseptic condition.

### Isolation of Actinomycetes

Ten grams of soil sample were taken in 95ml of water and placed in Shaker for 2 hours. The preparation is known as master dilution. From the master dilution actinomycetes were isolated by serial dilution technique and cultured on Starch casein Agar, International Streptomyces project Medium No. 2 (ISP2). All supplemented with Flucanazole (20mg $L^{-1}$) for the reduction of contamination caused by fungi (Labeda and Shearer, 1990). The plates were incubated at room temperature for 14 – 21days. The selected colonies were further streaked onto International Streptomyces project No. 2 Agar (ISP2). Thus isolated colonies were preserved in agar slants and glycerol stock and stored at -20˚c.

### Phytopathogenic Fungi

*R.solani* which causes damping off disease was isolated from the infected soil sample and *S.sclerotiorum* which causes stem rot was isolated from infected stem of the tomato plants. It is then identified in our lab and maintained in Potato Dextrose Agar (PDA) slants.

### Screening for Biocontrol Activity by Dual Culture Method

The isolates were evaluated for its antagonistic activity against two phytopathogens namely *R.solani* and *S.sclerotiorum* using dual culture technique (Huang and Hoes, 1976). The actinomycete culture was streaked in Starch Casein Potato Dextrose Agar (SCPDA) and incubated for 5 days, after which the mycelial plug of the fungi was streaked perpendicular to the actinomycete strain and incubated for 5 days. The results were observed for 14 days.

### Identification of the Antagonistic Actinomycete Strain

The potent actinomycete strain which showed good antagonistic activity was characterized by physiochemical and biochemical methods. Physiochemical methods consist of macroscopic and microscopic methods. The microscopic

characterization was done by cover slip culture method. The mycelial structure, colour and arrangement of the spore were observed through microscope. The observed structure was compared with the Bergey's manuel of Determinative Bacteriology and the organism was identified. Gram staining, Acid fast staining and various Biochemical tests, were performed.

Pigment production, starch hydrolysis, Casein hydrolysis, catalase test, Oxidase test and various biochemical tests such as Indole, Methyl red, Voges proskauer, citrate utilization test and Urease production Nitrate reduction tests were performed. 16s rDNA Sequencing was done for confirmation of the species. Effect of different temperature, Nacl concentration and pH were also observed. The Chitinase activity of AMP14 was also observed.

**DNA Isolation**

Overnight cultured microbial cells were harvested by centrifugation, and cell pellets were suspended in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) containing 2 mg of lysozyme per ml in a total volume of 5 ml. Samples were incubated at 30°C for 30 min, which was followed by addition of 1.2 ml of 0.5 M EDTA and protease (final concentration, 0.2 mg/ml) and an additional 30 min of incubation. After addition of 0.7 ml of 10% (wt/vol) sodium dodecyl sulfate (SDS), 1.8 ml of 5 M NaCl, and 1.5 ml of 10% CTAB-NaCl solution (10% [wt/vol] hexadecyltrimethyl ammonium bromide in 0.7 M NaCl), the mixtures were incubated at 65°C for 20 min. Samples were extracted with chloroform-isoamyl alcohol (24:1, by volume), and the supernatants were transferred to clean tubes. DNA was precipitated by addition of 0.6 volume of isopropanol and pelleted by centrifugation (48,000 x g, 15 min). DNA pellets were rinsed with 70% (wt/vol) ethanol, dried, dissolved in TE buffer, and quantified by absorption spectrophotometry at 260 nm.

**Universal Bacterial Primers and PCR Conditions**

Each PCR mix contained ~25 ng DNA, 10 pm each forward and reverse primer, 20 mM dNTPs (Invitrogen), 2.5 mM MgCl2 and 1.0 U Platinum Taq DNA polymerase (Invitrogen) in the manufacturer's buffer. All reactions were done with a 3 min 95 °C hot start followed by 35 cycles of 95 °C for 1 min, the primer-specific annealing temperature for 1 min, and 72 °C for 2 min, followed by a 30 min 72°C extension. Primers designed to anneal to conserved regions in actinomycetes were used to amplify bacterial 16S rDNA sequences that contained intervening species-specific regions. The forward primer, 8F (5'-AGAGTTTGATCCTGGCTCAG- 3'), was used with reverse primers 1492R (5'-GGGTTACCTTGTTACGACTT-3') with an annealing temperature of 56 °C and template DNA from the root-canal contents to amplify 1.3 and 1.4 kbp regions, respectively, of bacterial 16S rDNA (Baker et al., 2003). The products of primers 8F and 1391R and the products of primers 8F and 1492R with pooled DNA from asymptomatic patients as template, and the products of the same primer pairs using DNA template pooled from symptomatic patients, were cloned for sequence analysis. An equivalent number of clones were analysed from both the symptomatic and asymptomatic patients, for a total of ~1500 clones.

**Optimization of the Medium**

Various Carbon sources such as glucose, starch, arabinose, fructose, galactose, mannitol, maltose, sucrose, rhamnose and raffinose were given for growth and nitrogen sources such as peptone, yeast extract, L-tyrosine, tryptone, L-asparagine, potassium nitrate, L-histidine and L-threnonine were given and the growth is observed.

Different types of medium such as Starch Casein Agar, (International Streptomyces Agar medium ) Isp2, Isp4, Glucose Asparagine Agar, Tryptic soy agar, Oat meal Agar, Antibiotic Production Medium, Modified Potato Dextrose Agar, Malt extract Agar and Modified Isp2 were used for the optimization of the medium.

**Preparation and Screening of Antagonistic Effect of Crude Extract**

The strain AMP14 was found to be very effective and was selected to carry out further studies. It was grown in modified $ISP_2$ medium and kept in a shaker under 290 g at room temperature (28 ± 2ºC) for 14 days. After 14 days the culture filtrate was centrifuged at 10,000 g for 15 minutes and then extracted using ethyl acetate with a separating funnel and it was evaporated using rotary evaporator and dried. The dried crude extract was checked for their antagonistic effect against the two phytopathogens *R. solani* and *S. sclerotiorum* using well diffusion method.

The concentrated crude metabolites were mixed with silica gel (60-100 mesh) to prepare the metabolite-silica gel slurry and air-dried. The slurry was fractionated by silica gel column chromatography using glass column. Silica gel of 60 – 100 mesh was packed in the column and washed with hexane. Then the metabolites-silica gel slurry was loaded on to the column and were successively eluted initially with 100 % hexane followed by hexane: chloroform; chloroform: ethyl acetate; ethyl acetate : methanol and finally with methanol (100 %). About 100 ml of each of the solvent system was used for elution. The presence of the compounds was analyzed using thin layer chromatography (TLC). Based on the TLC analysis, further column was carried out using sephadex LH-20. The purified compound was further confirmed by TLC and UV, NMR, FTIR, GC-MS characterization.

## RESULTS

**Sample Collection**

Soil samples were collected from 5-15cm depth into sterile plastic bags from different rhizosphere soil of tomato fields from Kancheepuram, Villupuram and Dharmapuri districts and brought to the laboratory in aseptic condition and the pH of the soil samples were noted.

**Table 1: pH of Soil Samples Collected from Different Districts**

| Collection of Soil (Districts) | Soil pH |
|---|---|
| Villupuram | 7.2 |
| Dharmapuri | 7.8 |
| Kancheepuram | 5.8 |

**Isolation of Actinomycetes**

A total of fifty actinomycetes species were isolated from various rhizosphere (tomato) soils using different selective media such as Starch casein Agar, International Streptomyces Project Medium No. 2 (ISP2) and Glucose Asparagine Agar. They are designated as AMP-1 to AMP-50.

**Table 2: Colony Morphology of 50 Actinomycete Species Isolated from Different Rhizosphere Soils (Tomato)**

| Strain | Size | Shape | Nature | Appearance | Aerial Mycelium | Sub Mycelium |
|---|---|---|---|---|---|---|
| Amp 1 | Moderate | Concentric | Powdery | Pin drop | Grey white | Creamy |
| Amp 2 | Moderate | Concentric | Powdery | Pin drop | White | Brown |
| Amp 3 | Moderate | Concentric | Powdery | Pin drop | Pinkish white | White |
| Amp 4 | Moderate | Round | Powdery | Pin drop | Pinkish white | White |
| Amp 5 | Moderate | Round | Powdery | Flat | Grey | Red |

| | | | | | | |
|---|---|---|---|---|---|---|
| Amp 6 | Moderate | Round | Powdery | Flat | Pinkish white | Brown |
| Amp 7 | Moderate | Round | Powdery | Flat | Greyish white | Creamy |
| Amp 8 | Moderate | Round | Powdery | Flat | White | Brown |
| Amp 9 | Moderate | Irregular | Slimy with powder | Pin drop | Pinkish purplish powdery | Creamy |
| Amp 10 | Moderate | Round | Powdery | Flat | Brownish green | Green |
| Amp 11 | Smooth | Round | Powdery | Flat | Pin white | White |
| Amp 12 | Moderate | Round | Powdery | Pin drop | White | Creamy |
| Amp 13 | Smooth | Round | Powder slimy | Pin drop | White | Creamy |
| Amp 14 | Moderate | Round | Powdery | Flat | Brownish red | Red |
| Amp 15 | Moderate | Concentric | Powdery | Pin drop | Grey | Red |
| Amp 16 | Smooth | Round | Powdery | Pin drop | White | Brown |
| Amp 17 | Smooth | Round | Powdery | Pin drop | White | Brown |
| Amp 18 | Moderate | Round | Powdery | Flat | Pink white | Creamy |
| Amp 19 | Moderate | Round | Powdery | Pin drop | White | Brown |
| Amp 20 | Moderate | Concentric | Powdery | Pin drop | Grey white | Red |
| Amp 21 | Moderate | Round | Powdery | Pin drop | White | Green |
| Amp 22 | Smooth | Round | Powdery | Pin drop | White | White |
| Amp 23 | Smooth | Round | Powdery | Pin drop | White | Creamy |
| Amp 24 | Moderate | Irregular | Powdery | Pin drop | White | Creamy |
| Amp 25 | Smooth | Round | Slimy | Pin drop | Yellow cream | Yellow cream |
| **Strain** | **Size** | **Shape** | **Nature** | **Appearance** | **Aerial mycelium** | **Sub mycelium** |
| Amp 26 | Moderate | Round | Powdery | Flat | Pinkish white | Creamy |
| Amp 27 | Moderate | Round | Powdery | Pin drop | White | Cream |
| Amp 28 | Moderate | Round | Powdery | Pin drop | Grey | Red |
| Amp 29 | Moderate | Concentric | Powdery | Flat | Dull white | Creamy |
| Amp 30 | Smooth | Round | Powdery | Pin drop | White | Creamy |
| Amp 31 | Moderate | Round | Powdery | Pin drop | White | Brown |
| Amp 32 | Moderate | Round | Powdery | Flat | Pinkish white | Creamy |
| Amp 33 | Moderate | Round | Powdery | Pin drop | White | Cream |
| Amp 34 | Moderate | Concentric | Powdery | Pin drop | Pinkish white | White |
| Amp 35 | Smooth | Concentric | Powdery | Pin drop | Grey white | Creamy |
| Amp 36 | Moderate | Round | Powdery | Flat | Grey | Grey |
| Amp 37 | Smooth | Round | Powdery | Pin drop | White | Brown |
| Amp 38 | Smooth | Round | Powdery | Pin drop | White | Creamy |
| Amp 39 | Smooth | Concentric | Slimy | Pin drop | Yellow cream | Yellow cream |
| Amp 40 | Moderate | Round | Powdery | Flat | Whitish pink | Creamy |
| Amp 41 | Moderate | Concentric | Rough | Pin drop | Violet | Violet |
| Amp 42 | Smooth | Round | Powdery | Pin drop | Orange | White |
| Amp 43 | Moderate | Round | Powdery | Pin drop | White | White |
| Amp 44 | Moderate | Concentric | Powdery | Flat | Dull white | Creamy |
| Amp 45 | Smooth | Round | Powdery | Pin drop | White | Creamy |
| Amp 46 | Moderate | Concentric | Powdery | Pin drop | Grey | Red |
| Amp 47 | Smooth | Round | Slimy | Pin drop | White | Brown |
| Amp 48 | Smooth | Round | Powdery | Flat | White | Grey |
| Amp 49 | Moderate | Concentric | Powdery | Pin drop | White | Grey |
| Amp 50 | Smooth | Round | Powdery | Pin drop | Grey | Brown |

**Isolation of Phytopathogenic Fungi**

*R. solani* was isolated from infected plant and its immediate soil samples and

*S. sclerotiorum* were isolated from infected stem of tomato plants. It was identified in lab and was maintained on Potato Dextrose Agar (PDA) slants.

**Screening for Biocontrol Activity by Dual Culture Method**

The isolates were evaluated for its antagonistic activity against two phytopathogenic fungi namely *R. solani* and *S. sclerotiorum* using dual culture technique (Huang and Hoes, 1976). Among all the fifty isolates of actinomycetes tested, seven strains showed good antagonistic activity against *R. solani* and only one strain showed best antagonistic activity against *S. sclerotiorum.* The strain AMP14 showed very good antagonistic effect against the two phytopathogens and it is chosen for further analysis.

**Identification of the Antagonistic Actinomycete Strain**

The potent strain AMP14 was characterized by physiochemical and biochemical methods.

**Table 3: Physiological and Biochemical Characteristics of AMP14**

| TESTS | CHARACTERISTICS |
|---|---|
| Gram staining | Positive |
| Pigment production | Positive |
| Starch hydrolysis | Positive |
| Casein hydrolysis | Positive |
| Catalase test | Negative |
| Oxidase test | Negative |
| Indole production | Positive |
| Methyl red test | Positive |
| Voges proskauer | Positive |
| Citrate utilization test | Positive |
| Urease test | Positive |
| Nitrate reduction | Positive |
| $H_2S$ production | Positive |
| Chitin | Positive |

**16s rDNA Sequencing**

>Acti_16s_FWD_IX12

ACGCTGAAGCATCTTCGGGTGTGGATGAGTGGCGAACGGGTGAGTAACACGTGGGTAATC

TGCCCTGCACTCTGGGATAAGCCCGGGAAACTGGGTCTAATACCGGATAGGACACATGGC

CGCATGGTCTGTGTGTGGAAAGTTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTT

GTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGG

CCACACTGGGTCTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTGC

GCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGGGGGATGACGGCCTTCGGGTTGTA

AACCTCTTTCGACATCGACGAAGCCTTCGGGTGACGGTAGGTGTAGAAGAAGCACCGGCT

AACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGATTTATTGGG

CGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCGTTCGTGAAAACTGGAGGCTTAACCTTCA

GCTTGCGGTCGATACGGGCAGACTTGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAG

CGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGAT

ACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCAC

GCCGT

>Acti_16s_RvE_IX12

TCTGGGCCGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCC

TGGTAGTCCACGCCGTAAACGTTGGGCGCTAGGTGTGGGGACTGTTTCCACGGTTCCTGT

GCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAA

AGGAATTGACGGGGGCCCGCTCAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGA

AGAACCTTACCTGGGTTTGACATGCACTAGACTGCCTCAGAGATGGGGTTTCCCTTGTGG

TTGGTGTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTC

CCGCAACGAGCGCAACCCTTGTCCTGTGTTGCCAGCACGTAATGGTGGGGACTCGCGGGA

GACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCAACATGCCCCTTATG

TCCAGGGCTTCACANATGCTACAATGGCCGGTACAGAGGGTTGCGATGCCGTGAGGTGGA

GCGAATCCCTTAAAGCTGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGT

CGGAGTCGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTA

CACACCGCCNGTCACGTCATGAAAGTCGGTA

>Contig-0

ACGCTGAAGCATCTTCGGGTGTGGATGAGTGGCGAACGGGTGAGTAACACGTGGGTAATC

TGCCCTGCACTCTGGGATAAGCCCGGGAAACTGGGTCTAATACCGGATAGGACACATGGC

CGCATGGTCTGTGTGTGGAAAGTTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCAGCTT

GTTGGTGGGGTGATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGG

CCACACTGGGTCTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTGC

GCAATGGGCGAAAGCCTGACGCAGCAACGCCGCGTGGGGGATGACGGCCTTCGGGTTGTA

AACCTCTTTCGACATCGACGAAGCCTTCGGGTGACGGTAGGTGTAGAAGAAGCACCGGCT

AACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGATTTATTGGG

CGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCGTTCGTGAAAACTGGAGGCTTAACCTTCA

GCTTGCGGTCGATACGGGCAGACTTGAGTTCGGTAGGGGAGACTGGAATTCCTGGTGTAG

CGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGGGCCGAT

ACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCAC

GCCGTAAACGTTGGGCGCTAGGTGTGGGGACTGTTTCCACGGTTCCTGTGCCGTAGCTAA

CGCATTAAGCGCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACG

GGGGCCCGCTCAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTACC

TGGGTTTGACATGCACTAGACTGCCTCAGAGATGGGGTTTCCCTTGTGGTTGGTGTACAG

GTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC

GCAACCCTTGTCCTGTGTTGCCAGCACGTAATGGTGGGGACTCGCGGGAGACTGCCGGGG

TCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCAACATGCCCCTTATGTCCAGGGCTTC

ACANATGCTACAATGGCCGGTACAGAGGGTTGCGATGCCGTGAGGTGGAGCGAATCCCTT

AAAGCTGGTCTCAGTTCGGATCGGGGTCTGCAACTCGACCCCGTGAAGTCGGAGTCGCTA

GTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCNG

TCACGTCATGAAAGTCGGTA

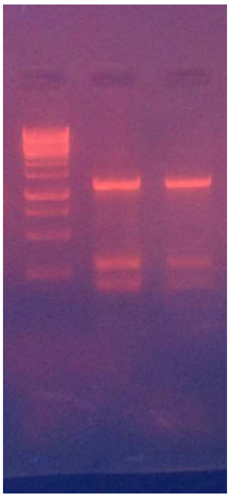

**Figure 1: 16srDNA Sequencing**

- Lane-1- 1 Kb ladder
- Lane-2- PCR product
- Plane3 PCR product

**Different Carbon and Nitrogen Sources**

Among the various carbon sources tried, maximum growth was observed in1mM/2% starch, galactose and glucose (Figure 2) and in nitrogen source peptone at 2mM/2% supported maximum growth of AMP14

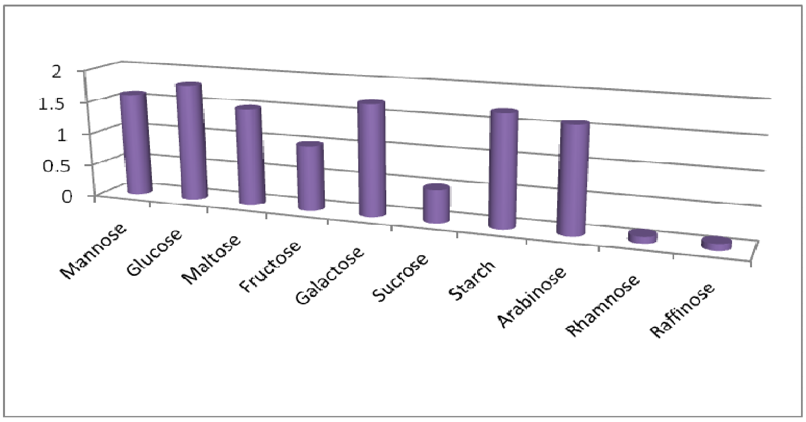


**Figure 2: Effect of Different Nitrogen Sources on the Growth of AMP14 Strain**

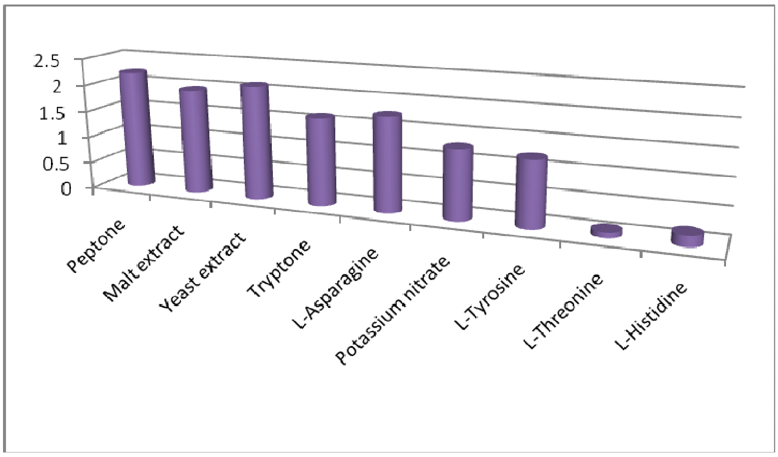


**Figure 3**

**Screening of Antagonistic Effect of Crude Extract**

The strain AMP14 was found to be very effective and was selected to carry out further studies. The dried crude extract which was checked for their antagonistic effect against the two phytopathogens *R. solani* and *S. sclerotiorum* using well diffusion method proved to be very effective against both the pathogens. It is subjected to thin layer chromatographic technique.

Based on the TLC analysis, further column was carried out using sephadex LH-20. The purified compound was further confirmed as Oleic acid and L-Ascorbyl palmitate by TLC and UV, NMR, FTIR, GC-MS characterization.

## DISCUSSIONS

The main purpose of this study is to control two phytopathogenic fungi such as *R. solani* and *S. sclerotiorum* which causes the dreadful disease such as damping off and Stem rot, which affects most of the plants which leads to mortality of those plants. The present work states the invitro potential of the selected actinomycete strain to control the above said dreadful fungal phytopathogens. The secondary metabolite production is found abundant in the AMP14 and it supported in curbing the growth of the two dreadful fungal pathogens *R. solani* and *S. sclerotiorum*. The compounds which are isolated from AMP14 such as Oleic acid and L-Ascorbyl palmitate also found to be very effective against the two fungal pathogens.

## CONCLUSIONS

The findings from this investigation reveals that the strain AMP14 was found to be potent which was the soil isolate of actinomycetes exhibited good antagonistic activity against the two phytopathogens to the other isolates of actinomycetes and also the isolate AMP14 produces good secondary metabolites and the compounds which supported in curbing the growth of both the fungal pathogens.

## ACKNOWLEDGEMENTS

One of the authors (S.B.P) thank Director, CAS in Botany for the laboratory facilities.

## REFERENCES

1. Baker, G. C., Smith, J. J. & Cowan, D. A. (2003). Review and reanalysis of domain-specific 16S primers. *J Microbiol Methods* **55**, 541–555.
2. Boland GJ, Hall R. 1994. Index of plant hosts of *Sclerotinia sclerotiorum. Canadian Journal of Plant Pathology* **16**: 93–108.
3. Champness, W.C. and Charter, K.F. 1994. Regeneration and integration of antibiotic producing and morphological differentiation in *Streptomyces spp. In:Regulation of bacterial differentiation*. (Eds. Piggot, J. Moran, C. and Youagaman, P.), American Society for Microbiology, Washington, DC, USA.
4. Charter, K.F. and Hopwood, D.A. 1993. *Streptomyces genetics. American Society for Microbiology,* WashingtonDC, USA.
5. ELAD, Y. Chet. I and Katan. Y. (1980). *Trichoderma harzianum* a biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani. Phytopathology* **70**: 119-121.
6. Goodfellow M. and Williams, S.T. (1983). Ecology of actinomycetes. *Ann. Rev. microbiol.* **37**: 189-216.
7. Goodfellow, M, S.T, Williams and M. Mordarski 1988. Actinomycetes in Biotechnology. *Academic press Limited, London.* 501p.
8. Goodfellow, M. and A.G. O' Donnewell, 1989. Search and discovery of significant Actinomycetes. In microbial products. New approach.44. *The symposium of the society for General Microbiology ed.* Baumberg, S. Hunter, I and Rhodes M. Cambridge, *U.K, Cambridge university press.* pp:343-383.
9. Hegedus DD, Rimmer SR. 2005. *Sclerotinia sclerotiorum*: when "to be or not to be" a pathogen*? FEMS Microbiology Letters* **251**: 177–184.
10. Heydarian, S.M., Ison, A.P., Lilly, M.D. and Ayazi Shamlou, P.A. 2000. Turbulent breakage of filamentous bacteria in mechanically agitated batch culture. *Chem. Eng. Sci.* **55**: 1775-1784.
11. Huang, M.C. and Hoes, J.A. 1976. Penetration and infection of *Sclerotinia sclerotiorum* by *Corinithurum minitans*. *Can. J. Bot*. **54**: 406-410.
12. Jensen, P. R., R. Dwight and W. Fenical. 1991. Distribution of actinomycetes in near-shore tropical marine sediments. *Appl. Environ. Microbiol*. **57**:1102-1108

13. Labeda, D. 1993. DNA relatedness among strains of *Streptomyces lavendulae* phenotypic cluster group. *Int. J. System. Bacteriol*. **43**:822-825.

14. LATORRE, Bernardo. *Enfermedades de las plantas cultivadas*. Santiago; Ediciones Universidad Católica de Chile. 2004. 638 p. ISBN 956-14-0756-6. (Ref)

15. Lechevalier, M.P., 1981. Ecological associations involving actinomycetes. Zentralblatt fur Bakteriologie, *Mikrobiologie Hygiene I Abteilung supplement* **11**, 159-166.

16. Neha Dev and A.Y. Dawande. 2010. Biocontrol of soil borne plant pathogen *Rhiozoctonia solani* using *Trichoderma* sp and *Pseudomonas fluorescens*. *Asiatic J. Biotech. Res.* **01**: 39-44 (Ref)

17. Williams, S.T., Wellington, E.M.H., 1982. Actinomycetes. In: page, A.L., Miller, R.H.) Keenchy, O.R. (Eds.), Methods of soil Analysis, Part 2, *Chemical and Microbiological properties, second society of America, Madison*, pp.969-987.